

added to co-culture of PBMC and colorectal cancer (colorectal adenocarcinoma) cell line.

[0046] FIG. 9F and FIG. 9G are graphs showing PD-1 receptor occupancy on CD8⁺ T cells and IFN- γ production respectively when anti-PD-1-TGFR2 fusion protein is added to co-culture of PBMC and head and neck cancer (pharyngeal carcinoma) cell line.

[0047] FIG. 9H and FIG. 9I are graphs depicting TGF- β 1 and TGF- β 2 concentration respectively in supernatant of PBMC and colorectal cancer (colorectal adenocarcinoma) cells co-culture in presence of anti-PD-1-TGFR2 fusion protein.

[0048] FIG. 9J and FIG. 9K are graphs depicting TGF- β 1 and TGF- β 2 concentration respectively in supernatant of PBMC and head and neck cancer (pharyngeal carcinoma) cells co-culture in presence of anti-PD-1-TGFR2 fusion protein.

[0049] FIG. 9L and FIG. 9M are graphs depicting IFN- γ production respectively in 3D spheroid culture of colorectal cancer (colorectal adenocarcinoma) cells or head & neck cancer (pharyngeal carcinoma) cells and PBMC in presence of anti-PD-1-TGFR2 fusion protein as compared to anti-PD-1 alone.

[0050] FIGS. 10A-10D show effect of anti-PD-1-TGFR2 fusion protein treatment on T cell proliferation and activation in the presence of recombinant TGF- β 1.

[0051] FIGS. 11A-11F show expression of various cytokines by PBMC in the presence recombinant TGF- β 1 and in the presence of anti-PD-1, anti-PD-1-TGFR2 fusion protein or control antibodies.

[0052] FIG. 12A shows the effect of anti-PD-1-TGFR2 fusion protein on tumor growth as compared to anti-PD-1 alone in a humanized mouse model of colorectal cancer.

[0053] FIG. 12B shows that treatment with anti-PD-1-TGFR2 fusion protein significantly increases CD8⁺ T cell to T_{reg} ratio in tumor in a humanized mouse model of colorectal cancer.

[0054] FIG. 12C shows the effect of anti-PD-1-TGFR2 fusion protein treatment on perforin expression levels as compared to anti-PD-1 treatment in a humanized mouse model of colorectal cancer.

[0055] FIG. 12D and FIG. 12E shows the effect of anti-PD-1-TGFR2 fusion protein treatment on TGF- β 1 and TGF- β 2 concentrations as compared to anti-PD-1 treatment respectively in a humanized mouse model of colorectal cancer.

[0056] FIG. 13A shows treatment with anti-PD-1-TGFR2 fusion protein significantly improved IFN γ production compared to anti-PD-1 treatment in an in vitro model of head and neck cancer. FIGS. 13B-13G show treatment with anti-PD-1-TGFR2 fusion protein significantly increases T-cell function as evidenced by expression analysis of various pathway genes.

[0057] FIG. 14A shows the effect of anti-PD-1-TGFR2 fusion protein on tumor growth as compared to anti-PD-1 alone in a humanized mouse model of head and neck cancer.

[0058] FIG. 14B shows survival of tumor bearing mice in humanized mouse model of head and neck cancer when treated with anti-PD-1-TGFR2 fusion protein vs. anti-PD-1 alone or isotype control.

[0059] FIG. 14C shows ratio of CD8⁺ T cells to regulatory T cells in tumors of mice treated with anti-PD-1-TGFR2 fusion protein in a humanized mouse model of head and neck cancer.

[0060] FIG. 14D and FIG. 14E shows the effect of anti-PD-1-TGFR2 fusion protein on TGF- β 1 and TGF- β 2 concentrations as compared to anti-PD-1 alone in a humanized mouse model of head and neck cancer.

[0061] FIG. 14F shows the effect of anti-PD-1-TGFR2 fusion protein on IFN- γ production in a humanized mouse model of head and neck cancer.

[0062] FIGS. 15A-15B shows the effect of anti-PD-1 (VH7/VL6)-TGFR2 fusion protein on IFN- γ production and TGF- β 1 concentration in primary colorectal cancer patient samples.

[0063] FIG. 15C shows the gene expression analysis of primary colorectal cancer patient samples co-cultured with anti-PD-1 (VH7/VL6)-TGFR2 fusion protein.

[0064] FIG. 16 shows a graph depicting the results of a cytotoxicity assay of anti-PD-1 (VH7/VL6)-TGFR2 fusion protein compared to anti-PD-1-TGFR2 fusion protein.

[0065] FIG. 17 is a graph depicting the results of a cytotoxicity assay of anti-PD-1 (VH7/VL6)-TGFR2 fusion protein in combination with a chimeric receptor antigen (CAR) T cells vs. anti-PD-1 in combination with a CAR T cells vs. CAR T cells alone.

[0066] FIG. 18A and FIG. 18B are graphs depicting the results of a cytotoxicity assay of anti-PD-1 (VH6/VL5)-TGFR2 fusion protein in combination with CD33 CAR-T and a cytotoxicity assay of anti-PD-1 (VH7/VL6)-TGFR2 fusion protein in combination with CD33 CAR-T respectively.

[0067] FIG. 19A and FIG. 19B are graphs depicting tumor cell lysis using anti-PD-1 (VH6/VL5)-TGFR2 fusion protein and anti-PD-1 (VH7/VL6)-TGFR2 fusion protein respectively when co-cultured with NK cells.

[0068] FIG. 20 is a graph depicting Biacore analysis of simultaneous binding of TGF- β 1 and PD-1 by anti-PD-1 (VH6/VL5)-TGFR2 fusion proteins using different linkers.

[0069] FIG. 21 is a graph showing blockade of PD-1/PD-L1 interaction by anti-PD-1 IgG4-ADA2.

[0070] FIG. 22 is a graph showing ADA2 enzymatic activity measured for anti-PD-1 hIgG1-ADA2 and anti-PD-1 hIgG4-ADA2.

[0071] FIGS. 23A-23C are graphs showing the effect of various variants of anti-PD-1 and anti-PD-1-ADA2 fusion proteins on PD-L1/PD-1 interaction.

[0072] FIGS. 24A-24F are graphs showing the enzymatic activity of various variants of anti-PD-1-ADA2 fusion proteins as measured by ADA enzymatic activity.

[0073] FIG. 25 is a graph showing the enzymatic activity of anti-PD-1-mutADA2 vs. anti-PD-1-wtADA2 as measured by ADA enzymatic activity.

[0074] FIG. 26A-26D are graphs depicting the effect of variants of anti-PD-1-wtADA2 on T cell proliferation.

[0075] FIG. 26E is a graph depicting IFN γ production by anti-PD-1-wtADA2 as compared to anti-PD-1 or isotype control.

[0076] FIGS. 27A-27B are graphs depicting the effectiveness of wtADA2 and mutADA2 to reverse adenosine-mediated suppression of T cell proliferation.

[0077] FIG. 28 is a graph depicting the effect of variants of anti-PD-1-ADA2 fusion proteins on the blockade of PD-1/PDL1 interaction.

[0078] FIG. 29 is a graph showing the enzymatic activity of anti-PD-1-ADA2-scFv-Fc as measured by ADA enzymatic activity.